THE CURRENT STATUS OF THE DEVELOPMENT OF ANTIMICROBIAL AGENTS*

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IT is a privilege indeed for me to have been invited to discuss this evening the current status of the development of antimicrobial agents. To consider how far one has advanced in any field of endeavor is invariably worthwhile, for it forces one to give consideration also to that which has not been accomplished.

Much of what I shall say tonight will be historical in nature, for, as pointed out recently by a well-known scientist:

> "The past underlies the present, qualifies and moderates it, in some ways limits it and in some ways enriches it. . . .
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> "It is a recurrent experience of scientific progress that what was yesterday an object of study, of interest in its own right, becomes today something to be taken for granted, something understood and reliable, something known and familiar – a tool for further research and discovery."[1]

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Like many developments in science, knowledge of the antimicrobial agents has grown in haphazard fashion. Isoniazid (isonicotinic acid hydrazide), one of the most powerful antimicrobial agents in use today, was first synthesized by Meyer and Mally[2] in 1912. By that time, sulfanilamide already had been synthesized by Gelmo in 1908.[3] Paul Ehrlich already had called attention to the potentialities of chemotherapeutic agents, and microbiologic techniques for their study already had been well established by Pasteur, Koch, and others. It was not until a quarter of a century later, however, that attention was focused specifically on evaluation of the potential significance of sulfanilamide, its

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derivatives, and other similarly active compounds. It was not until forty years later that the chemotherapeutic properties of isoniazid were demonstrated.

Two decades have now elapsed since the chemotherapeutic activity of Prontosil rubrum was first described by Domagk[4] and since Levaditi and his associates[5] first demonstrated that the para-aminobenzene sulfonamide group breaks away from the rest of the Prontosil molecule in vivo, and is responsible for its chemotherapeutic properties. The subsequent synthesis of an extensive series of related compounds and definition of the scope and limitations of these agents, coupled with the timely observations of Dubos[6] on the action of gramicidin, served as a stimulus to Florey and his co-workers[7, 8] in their studies of the action of penicillin. Together, the observations made on these compounds introduced chemotherapy on a sound basis into medical practice.

The results obtained with the sulfonamides unquestionably were dramatic in nature. The remarkable effects achieved with penicillin, however, and the announcement of the discovery of streptomycin within a few years after its introduction, placed emphasis upon the prevalence of antibiotic-producing microorganisms in nature. Interest in biologic sources of these compounds was in no way diminished by the early difficulties encountered in the production of penicillin on a large-scale basis. In the search for additional compounds with chemotherapeutic properties, attention was thus focused for almost ten years thereafter upon those species of microorganisms which could be cultivated in quantity. Efforts in this direction were rewarded by the discovery of the tetracycline group of antimicrobials and numerous other compounds with specific although more limited action. It was perhaps the success of the carefully designed large-scale microbiologic studies, conducted during these years, that drew attention away from the field of synthetic chemicals. It is the success of these studies, perhaps, that accounts for our being largely dependent, in the year 1954, upon chemotherapeutic agents produced by organisms occurring in nature, although fully aware of the potentialities of synthetic chemicals.

One may justly wonder, at this time, if progress in the field of chemotherapy perhaps has been retarded by our having placed so much emphasis on those agents produced by living microorganisms. If as much emphasis had been placed, during the past ten years, upon evaluation of the chemotherapeutic properties of known or easily synthesized

chemical compounds, would greater strides have been made toward the control and eradication of infectious disease? Will the presently accepted and highly effective antibiotic agents be replaced in the future by synthetically-derived chemicals, as quinine has been replaced by chloraquin and primaquin?

It is of interest, perhaps, that, in the year 1953, the production, within the United States, of antibiotics alone totalled 2.1 million pounds, while the total sales exceeded 1.85 million pounds. According to a recent preliminary report of the United States Tariff Commission,[9] this represents a sales value of over 251 million dollars. According to this same report, an additional 4.7 million pounds of sulfa drugs, with a sales value of nearly 17 million dollars, likewise were produced during this same year. These figures do not include isoniazid, para-aminosalicylic acid, the sulfones, or other such synthetically-produced compounds. If these had been included, the total sales value of specific antimicrobial agents, within the United States alone, might well have approached 300 million dollars. By way of comparison total medicinal sales within the United States during 1953 amounted to 54.2 million pounds, with a total dollar value of 409.1 million.

The fact that such quantities of the antimicrobial agents were produced and marketed within a single year is indicative of the position which they have assumed in the health and economy of our nation. The ready acceptance of such tremendous amounts is in a sense a measure of their established usefulness.

Production figures are not available for most countries other than the United States. It is known, however, that the production of antibiotics has spread to the four corners of the world, and, while the United States is still the leading manufacturer of these products, they are now being fermented on all five continents. In the Western Hemisphere, private firms are fermenting penicillin in Brazil, Argentina and Canada. In Europe, the United Kingdom is producing about double its home consumption and exporting the remainder all over the world. Indeed, it has been reported that 97,360 billion international units of penicillin, i.e., equivalent to 128,670 pounds, were produced in Great Britain during 1953.[10] The German pharmaceutical industry has shared in the rapid recovery of the whole German economy and it now produces penicillin and streptomycin for export as well as for its local needs. Both Italian and French pharmaceutical companies, furthermore,

have taken up antibiotic fermentation on a large scale, while the Scandinavian countries are represented in the picture with Sweden and Denmark also producing penicillin. In the Far East, Japan is becoming a major exporter of antibiotics, while penicillin fermentation is being undertaken not only by private companies but also by government laboratories in countries such as India and Australia. Indeed, in the latter country, chloramphenicol likewise is produced currently. Behind the "iron curtain," there are five plants about which we have received news – two in Poland, one in Hungary, one in Rumania, and another in East Germany which are producing chloramphenicol.

In addition to those antibiotics actually produced in foreign countries, however, exports of antibiotics from the United States to other countries totalled, in 1953, more than 310,000 pounds, with a sales value of over 90 million dollars.[11] They reached during the past year essentially all countries except those within the "iron curtain." It is unfortunate, however, that, despite the efforts of the World Health Organization and other similar agencies, economic conditions do not permit the antimicrobials to exert their full effectiveness in those very areas where the need for them is greatest. While economic conditions in many countries undoubtedly are influenced in large part by the health of the people of that nation, local taxes make the antimicrobial drugs prohibitive, generally increasing the cost to double that in the United States. Governmental regulations, local beliefs and customs, and at times superstition, furthermore, restrict their usage.

The development and large-scale production of antimicrobial agents may be considered today to be "an art," – a skill dependent upon practice and performance and acquired by experience, study and observation. Insofar as the antibiotics are concerned, increased knowledge concerning strain mutations has contributed significantly to their availability. The application of engineering principles to the design, developmental and operating phases of fermentation processes has accounted, however, in far greater measure for the phenomenal increase in their output.

Penicillin, streptomycin and dihydrostreptomycin, tetracycline, oxytetracycline, and chlortetracycline, chloramphenicol, the sulfonamides, isoniazid, and para-aminosalicylic acid are among the most widely used antimicrobials today. In addition, neomycin, polymyxin B, bacitracin, erythromycin and carbomycin, viomycin, tyrothricin, stil-

bamidine, and the sulfones are used in special circumstances. Mention should be made also of pyrazinamide for, although not widely available at present, frequent reference will be made to it in the present discussion.

The sulfonamides, isoniazid, para-aminosalicylic acid, the sulfones, stilbamidine, and pyrazinamide are produced by chemical synthesis only. The chemical structures of penicillin, streptomycin, chloramphenicol, and the three tetracyclines furthermore have been described, while chloramphenicol, originally produced through a fermentation process, has now been synthesized chemically and indeed is produced commercially by a synthetic process.

The antimicrobial agents which are in use today are essentially ones that "happened to be effective"; they came into clinical use through the process of "survival of the fittest." No information was available originally to explain why these, and not others, were effective. Despite the fact that the element of chance strongly influenced both their discovery and the development of our knowledge concerning them, however, certain principles have been established, and certain concepts introduced. Today, we find ourselves attempting to evaluate these principles and concepts, and attempting, in a very human and totally inadequate manner, to introduce rationality into a problem that perhaps will always defy rationalization.

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It has been recognized for some time that the two basic principles of antimicrobial therapy undoubtedly are: 1) *To be effective, an antimicrobial agent must be capable of exerting its growth inhibiting action in vivo against the specific microorganism concerned;* and 2) *once within the body, it must reach the site at which the microorganism resides in sufficient concentration and must remain there for a sufficient period of time to permit it to exert its effects.* Many factors, however, operate to prevent a drug from acting in vivo, and evaluation of those hurdles which a good chemotherapeutic agent must surmount in order to survive is essential before a rational approach to chemotherapy can be introduced.

Of prime importance is the fact that susceptibility of a given microorganism to a given antimicrobial agent in vivo is a necessary prerequisite for chemotherapeutic effectiveness. Organisms resistant to a given agent

in vitro are unlikely to be susceptible in vivo unless, as was the case with Prontosil rubrum, that compound is converted within the host to another substance capable of inhibiting growth of the microorganism in question. A compound exhibiting a significant degree of antimicrobial activity in vitro, on the other hand, may or may not show similar activity within the host. The degree to which in vitro and in vivo effects correspond varies with the compound in question, and is dependent in large part upon its pharmacologic properties. Susceptibility of an organism to a given agent is an essential prerequisite; it is not an adequate prerequisite for drug effectiveness.

The notion that bactericidal action in vivo may be essential to fully efficient antimicrobial therapy is currently receiving widespread consideration. It is difficult, of course, to estimate the degree of bacteriostatic or bactericidal action that may result within the host. Furthermore, one cannot state with certainty the extent to which an antimicrobial agent may be responsible for the effect observed, or the extent to which the resistance of the host may influence the end result.

In those instances in which control of an acute infection by an antimicrobial agent has implied to the casual observer that the invading microorganisms have been eradicated by that antimicrobial, actual evidence to this effect has not been forthcoming. In this regard it is of interest, perhaps, that extensive study of morphologically healed tuberculous lesions, removed from patients who had received chemotherapy for periods ranging from four to twelve months, has indicated that tubercle bacilli remain viable in the majority of instances, and that the viability of these organisms can be demonstrated.[12] Undoubtedly not all of the bacilli present in such lesions are viable; nonetheless, a portion is alive. They can be cultivated in vitro, and presumably they could multiply in vivo and bring about relapse under appropriate conditions. The evidence with respect to this particular infection emphasizes the fact that it is seldom, if ever, possible to eradicate every single member of a particular infecting microbial population.

The very fact that chemotherapy merely slows or halts multiplication of the microorganism implies that the success of chemotherapy must depend upon the ability of the patient to control the infection after the administration of the drug has been discontinued. Chemotherapy may speed the process of healing, or may increase the frequency of success in an individual; it cannot alter the basic mechanism of healing.

Various factors which conceivably may act to prevent sterilization of lesions within the host have been discussed recently by McDermott,[13] who has offered three possible explanations for the phenomenon. It is of interest to consider these in detail, for in so doing attention is focused upon those notions and concepts which reflect the extent of present knowledge concerning the action of antimicrobial agents in vivo. Many of the points which will be mentioned may well have been discussed previously during this Fortnight. They will be mentioned again at this time, however, in order to show how they relate to each other and perhaps contribute to the gradual development of a rational understanding of chemotherapeutic action.

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Failure to sterilize lesions may be due to the fact that:

1. *Certain of the parasites are so situated within the body that they are not reached by the antimicrobial drug:* As stated previously, abundant evidence is available to indicate that an antimicrobial agent will be effective in vivo only if it reaches the site of the invading microorganism in a concentration sufficient to inhibit its growth. An understanding of the pharmacologic action of a drug, therefore, is essential in evaluation of its degree of chemotherapeutic activity and in evaluation of the manner in which it can most effectively be administered.

Experience with penicillin, more than with any other agent, has served to emphasize the variable pharmacologic effects that may be obtained with different forms of a single substance, and the extent to which distribution of a drug within the body may be influenced by alterations in the molecule, or by use of appropriate carrying vehicles. For example, it is well known that the rapid absorption and excretion of sodium or potassium penicillin G when administered in aqueous solution, although at times advantageous in that peak concentrations are reached in the blood within a few minutes, makes administration of the drug difficult in many instances. This rapid absorption is due in large part to the high solubility rate of these particular penicillin salts. Procaine penicillin, in contrast, is a salt which is much less soluble in water, and long-sustained concentrations of penicillin are obtained in the blood and urine following administration of this form of the antibiotic. In like manner, the sparingly soluble salt, benzathine (N, N^1-dibenzyl ethylenediamine dipenicillin), is released slowly from tissue

depots, with resultant prolonged levels of penicillin in the blood following its administration. If a substance is rapidly absorbed, the normal process of excretion may reduce its effectiveness. Conversion to a less soluble form, however, may increase its chemotherapeutic potentialities, while control of crystal or particle size may further regulate the rate of solubility and thus the speed with which it is released into the general circulation. Through an understanding of the chemical and physical properties of a compound one may enhance significantly its potentialities.

To date, *only* those antimicrobial agents capable of distributing themselves throughout the extracellular fluids of the body and capable of exerting demonstrable activity within such fluids have come into widespread use. In general, these compounds have been most effective in the control of acute microbial infections in which the invading microorganisms have been located in readily accessible areas within the host. In such instances, in vivo susceptibility of the invading microorganism to an antimicrobial agent has generally been a sufficient basis for the assumption that a therapeutic response would be achieved with that antimicrobial, provided adequate dosage was used. Distribution of the drug throughout the extracellular fluids of the body with measurable concentrations in the blood presumably has created in such persons a situation in which the organisms were as vulnerable to attack by the antimicrobial as they would have been in an in vitro system.

The two cardinal principles of antimicrobial action, namely in vivo susceptibility of the invading microorganism to the agent concerned, and distribution of adequate quantities of that agent to the site at which the organism resides, imply that in some situations distribution of the drug throughout the extracellular fluids of the body may not be sufficient.

In chronic types of infection, the invading microorganisms generally are located in areas of necrosis. The ability of streptomycin and isoniazid to penetrate into necrotic lesions has been demonstrated by microbiologic assays on resected tissue, but no such data are available with respect to other antimicrobial substances. Unquestionably, an antimicrobial will be fully effective in the control of suppurative lesions only if capable of penetrating into necrotic tissue; and, what is probably of greater importance, it will be fully effective against such lesions only if capable of exerting full antimicrobial activity in the presence of necrotic tissue. Since many bacilli often exist within monocytes, it is apparent, furthermore, that an antimicrobial agent will be fully effec-

tive in the control of such infections only if capable of traversing the cell boundary. Indeed, it is not unreasonable to assume from the data presented by Mackaness[14] with respect to antituberculous agents that the relative activities of antimicrobial agents in the control of those infections in which microbial cells may exist within monocytes may be related in very large part to the ease with which they traverse cell boundaries and to the degree of activity which they can exert in an intracellular environment.

2. *That certain members of the microbial population are subsisting at such a low level of metabolic activity throughout the entire period of chemotherapy that they are physiologically insusceptible to the action of the drug:* It is unfortunate indeed that relatively little is known concerning the exact mechanisms by which antimicrobial agents exert their effects against individual microorganisms. Knowledge concerning the biochemical and enzymatic processes responsible for bacterial growth and reproduction has increased rapidly in recent years, and numerous studies have been conducted to determine the effect of antimicrobial agents upon these processes.[15] Of particular significance in this respect are the recent studies by Wisseman and his associates[16] which indicate that the introduction of chloramphenicol into a rapidly growing culture of Escherichia coli is followed by abrupt cessation of protein synthesis, although ribonucleic acid and desoxyribonucleic acid continue to be synthesized at an unaltered rate after protein synthesis has ceased. As pointed out by Wyss,[15] however, and as shown so clearly by the studies of Wisseman and his associates, when a cell stops growing, some cell reactions continue, but many of them stop; the problem is to determine which stoppages are causal and which are resultant of the growth inhibition. Unquestionably, however, the vast majority of antimicrobial agents studied to date are most active against rapidly metabolizing cells, and conditions which slow the metabolic rate may simultaneously diminish antimicrobial activity.

Dubos,[17] in a recently published book discussing the "Biochemical Determinants of Microbial Diseases," has summarized various factors within necrotic tissue that conceivably may suppress the metabolic activities of at least certain species of microorganisms and thus prevent an antimicrobial agent from acting against them. Low oxygen tension, local acidity, the presence of free fatty acids and other antimicrobial agents liberated by autolytic processes during necrosis, as well as other

factors, contribute to the development of an environment capable only of suppressing metabolic processes.

McDermott and Tompsett[18] recently reported that pyrazinamide and nicotinamide are highly active tuberculostatic agents when tested at the pH of inflammatory tissue, e.g., pH 5.0 to 5.5, but that these compounds show virtually no activity when used within the so-called physiologic ranges of pH. According to these investigators, the pyrazinamide and nicotinamide activation in the acidic environment, with no demonstrable activity at neutrality, is a distinctly unusual phenomenon among the antibacterial drugs which have been in general use. None of the others tested, with the possible exception of penicillin,[16] exhibits its maximal activity when the environment becomes more acid.

In 1944, at the Seventeenth Graduate Fortnight held at this Academy, Dubos,[19] in a discussion of the mode of action of chemotherapeutic agents in vitro, commented:

> "Another factor worth considering in analyzing reasons why so many antibacterial agents are ineffective on infected wounds or burns is that the reaction of these tissues is often more acidic than that of the blood. It is a known fact, on the other hand, that most antibacterial agents become less active as the pH is lowered. One may wonder, therefore, whether the problem of infected wounds and burns will not demand that attention be directed toward a class of substances exhibiting an optimum zone of activity slightly on the acid side."

Only now is this important fact receiving consideration. As stated by McDermott and Tompsett,[18] however:

> ". . . from what little is known about the biochemical environments of infectious processes, it is clear that they are quite complicated and probably subject to rapid changes in character. Consequently, although the biochemical environment of the lesion is probably responsible for most chemotherapeutic failures, it is unlikely that any one environmental factor, such as the degree of acidity, will represent the sole determinant of the antimicrobial activity of a drug."

The evidence unquestionably is inadequate. Insufficient is known concerning the mechanism by which antimicrobial agents act specifically to inhibit growth of microbial cells; insufficient is known concerning the factors within the host that may interfere with such inhibi-

tory effects. Nonetheless, based on that evidence which does exist, it seems highly unlikely that any of the currently available antimicrobial agents, except possibly pyrazinamide, – even if capable of penetrating into necrotic tissue, – would be fully active against microorganisms located therein. This remains one of the major unsolved problems in the field of antimicrobials today, and imposes an important limitation upon their scope.

3. *That the microorganisms which survive represent the more drug-resistant fraction of the total infecting population:* In 1944, Bigger[20] reported that penicillin may fail to sterilize large populations of staphylococci in vitro because of the presence of so-called "persisters," i.e., organisms which are not undergoing cellular division and against which penicillin, therefore, is capable of exerting little or no effect. It was suggested that, in some instances, failure to cure staphylococcal infections in man with penicillin might be due to the presence of similar "persisters" in vivo. Such cells were estimated to be few in number, rarely exceeding one per million bacteria.

Other investigators[21, 22] have similarly observed small numbers of organisms, within otherwise susceptible strains, *not* inhibited by penicillin and yet with penicillin-susceptible progeny.

The difference between the so-called "persisters" and resistant cells is, in part, that the former, on subculture, give rise to drug-susceptible progeny, while the latter give rise to resistant cells; it is in part a quantitative difference. Whereas the former exist in small numbers and merely prevent total sterilization, the latter readily increase in number and interfere also with the degree of bacteriostatic effect observed.[23] The eradication, therefore, of "persisters" presents a totally separate problem than does the control of drug-resistant strains.

It is scarcely necessary to comment on the emergence of drug-resistant strains. It is well known that microbial strains may be susceptible to one or more antimicrobial agents, they may be naturally resistant to such agents, or they may be resistant due to previous contact with the antimicrobial. Unquestionably, resistance develops more rapidly to some agents than to others, but perhaps of greater importance, is the fact that emergence of drug resistance is more frequent among some species of microorganisms than others. Indeed, what evidence there is at present suggests that quite possibly resistance becomes a problem only when dealing 1) with those microorganisms capable of adapting themselves

readily to growth under various or adverse circumstances, or 2) with those species which are difficult to identify by serological or biochemical means and perhaps may be considered to be "impure" in the sense that the population within a single strain conceivably may be comprised of representatives of various strains. Emergence of resistance has rarely, if ever, been noted among those microbial species with the most fastidious growth requirements, e.g., Streptococcus hemolyticus, Diplococcus pneumoniae, etc.

It is of interest in this regard to recall, perhaps, that each discovery in medicine uncovers a problem. Just as the introduction of the sulfonamides and penicillin uncovered the disease, primary atypical pneumonia, so also the widespread use of streptomycin, isoniazid, and para-aminosalicylic acid has led to recognition of the possible pathogenic significance of certain "atypical acid-fast bacilli" while the widespread use of the so-called "broad-spectrum antimicrobial agents" has aroused speculation concerning the nature and pathogenicity of staphylococci and certain other microbial species.

The appearance of resistant strains in vivo has been associated in large part with changes in microbial flora resulting within the host from administration of the antimicrobial agents. The occurrence of similar changes in flora, when species of microorganisms present in a given area are eliminated, is a natural phenomenon in nature. The new population, present after antimicrobial therapy, does not necessarily consist of drug-resistant variants of the organisms originally present in the individual patient's flora; rather, it consists of organisms which presumably were previously incapable of competing against those normally present within the host but now are able to grow and multiply in the absence of such competing cells.

It is reasonable to assume that occasionally a newly established organism may possess some degree of pathogenicity. *Only in rare instances,* however, have such microorganisms, – newly introduced into a host and/or increasing to predominant numbers within the host as a result of antimicrobial therapy, appeared to be pathogenic. In these few instances, surgical intervention generally has been associated with systemic invasion by the microorganisms, and it is not unlikely that factors associated with trauma may be essential to entry of these organisms into the body and to subsequent establishment of systemic infection. The nature of these factors has not been determined. It is of interest

in this regard, however, that Hammond and her associates[24] have described a strain of Pseudomonas aeruginosa, which on oral inoculation is quite harmless for mice, but which is rapidly fatal in irradiated mice infected on the eleventh day post irradiation. Since susceptibility to the infection is less on the fifth day post irradiation, and least of all directly after irradiation, and since damage to the intestinal mucosa is maximal within the first few hours after irradiation, these investigators conclude that the increased susceptibility of these animals is *not* due to increased permeability of the mucosa of the gut, but to impairment of the animal's natural defenses against infection.

Diverse opinions exist as to the long-range significance of the emergence of microbial strains resistant to the presently available antimicrobial agents. Nonetheless, as recently pointed out by Dowling,[25] a resistant strain, although of considerable importance to the individual harboring it, undoubtedly must be transmitted to other susceptible hosts if it is to assume larger significance.

> "The number of resistant bacteria in any given community will be directly proportional to the number of carriers of resistant bacteria, the number of susceptible hosts, and the frequency and intimacy of contact between the two. The spread of resistant strains may be prevented by the use of proper isolation technics or by raising the resistance of the host to the microorganism concerned."

One might add that the spread of resistant strains may be prevented also by use of antimicrobial agents only when there is a specific indication for such therapy, and then only in full therapeutic dosages. The currently prevalent trend to administer these drugs for prophylactic purposes is one which may well lead to unfortunate circumstances in the future.

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In summary, therefore, we find that the past ten to fifteen years have seen the introduction of large numbers of antimicrobial agents capable of controlling infectious diseases produced by a wide variety of pathogenic microorganisms. These ten to fifteen years have seen the development of methods for producing these agents within the United States in quantities sufficient to treat well over 100 million patients yearly, while the manufacture and distribution of these agents has been

extended throughout the world. During these ten to fifteen years, the mechanisms by which these agents exert their effects, both in vitro and in vivo, have been partially elucidated, and the conditions under which they may fail to act to some extent recognized.

The events of the past decade have brought us to a point at which one can no longer deny or question the value of these compounds as chemotherapeutic agents, when used either singly or in combination in appropriate situations. Yet they have brought us also to a point at which it is recognized that the limits of chemotherapy unquestionably are set by the amount of tissue destruction that has taken place before drug therapy is started, and by the sterilizing capacity of the drug or combination of drugs used. It is now a well accepted fact that the limits of chemotherapy can be extended only by the defense mechanisms of the body and/or by surgical intervention. It is with this in mind that Smadel, Woodward and their associates[26] are investigating the relation of the immune response to antimicrobial effects, while resectional surgery is widely used today in the treatment of tuberculosis. Quite probably it is for this reason also that the future will lie in attempts, already being made at least on an experimental basis, to alter the nature of the lesion by means of hormones, enzymes, or other such substances.

No mention has been made concerning the growth stimulatory properties of the antimicrobial agents. Of considerable economic importance, however, is the fact that minute amounts of penicillin, chlortetracycline, oxytetracycline, and bacitracin are capable of stimulating growth rates in poultry, while oxytetracycline and chlortetracycline are effective in swine. Many of the antimicrobial agents are capable likewise of stimulating growth rates of plants, and attempts are being made currently to elucidate the manner in which these effects are brought about.

Time does not permit comment concerning the various other uses being developed for the antimicrobial agents. The wide variety of conditions under which they may exert their growth inhibitory or growth stimulatory properties, however, continues to encourage the search for new ones.

In the development of new antimicrobial agents, the element of chance, once so important in antibiotic research, still influences the initial isolation of a strain capable of producing an antimicrobial sub-

stance; it influences the selection of the proper group of chemical compounds for study and evaluation. Techniques for the rapid testing of the antibiotic-producing properties of microorganisms, and knowledge concerning the growth requirements and optimum environmental conditions for growth of these organisms, however, has facilitated the search for new compounds. Paper chromatography, a technique designed to separate minute quantities on specially treated filter paper strips, allows rapid detection of new compounds or different forms of the same substance. By this technique, the identities of compounds may be established and their activities indicated even before isolation and purification are possible. Refined methods similarly facilitate the recovery and purification of those compounds warranting production in quantity, while infra red, ultra violet absorption, and Craig counter current distribution techniques aid in the analysis of the fractions isolated.

Experience with penicillin, streptomycin, the tetracyclines, and the other antimicrobial agents has established techniques for evaluation of new ones, and baselines for comparison. Despite this, however, speculation strongly influences one's interpretation of the potentialities of a compound, as suggested by experimental observations. The problem today is to choose among the many active compounds isolated. Not all can be produced on a large scale, for there is competition for fermentation tanks, for equipment for their recovery or synthesis, for animals and indeed suitable patients for their evaluation. One must select those that will be studied extensively and the very notions and concepts which we have discussed tonight form the basis on which the decision must be reached today. Without doubt, these notions at times may lead to erroneous decisions, and the element of chance is eliminated only insofar as our understanding of the mechanism by which these agents act in vivo may be correct.

In evaluating a new antimicrobial agent today, it is not enough to know that it is capable of exerting chemotherapeutic effects. Instead, one must ask: How effective is it in comparison with other available agents? Can it act against microorganisms located within necrotic tissue, as well as in the blood and extracellular fluids? Is it fully effective against microorganisms located intracellularly? Is it less likely to cause side reactions than other compounds covering the same disease spectrum? By what route must it be administered, and what will be its cost to the patient per day? If not suitable for systemic administration to

humans, does it have potentialities as a topical agent, or does it have industrial or agricultural uses? If not powerful enough to act alone, can it act synergistically?

The problem today is no longer a simple one. Furthermore, it is no longer centered merely on increasing the number of antimicrobial agents available. It is centered rather on "tailor-making" them to fit a specific need. Just as demonstration of the chemotherapeutic action of the sulfonamides stimulated interest in the action of penicillin, so also the discovery of many antimicrobial agents in nature has stimulated the desire to synthesize them chemically and to seek out those biochemical and enzymatic processes through which they exert their effects.

Unquestionably time and experience will clarify our understanding of these compounds and of the manner in which they act, either in an in vitro environment or within the host, to diminish or to stimulate growth rates. At present, one can only speculate.

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Candidates must be male interns or residents in Municipal or Voluntary hospitals in New York City.

The Committee stipulates that the study be pursued abroad, preferably in Great Britain or Continental Europe. The course of study, the auspices under which it is to be taken and the place or places where the study is to be pursued must receive the approval of the Committee.

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